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14. ABSTRACT Final report detailing effort to develop a capillary electrophoresis (CE) microchip sensor for the real-time, sensitive and selective detection of energetic explosives in seawater. Two primary issues are specifically addressed: 1) the development of new methods for enhancing the sensitivity to explosives via a pre-concentration step from seawater into the nonaqueous solvent, acetonitrile, and subsequent electrochemical detection following their microseparation on a microchip; and 2) methods for increasing the speed of analysis by employing the ultra-miniaturized solid phase extraction step that is coupled to a high speed microchip separation platform.						
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FINAL REPORT

GRANT #: N00014-03-WX-20118

PRINCIPAL INVESTIGATOR: Dr. Greg E. Collins

INSTITUTION: Naval Research Laboratory

GRANT TITLE: Explosives Detection in Seawater on a Microchip

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OBJECTIVE: To develop a lab-on-a-chip sensor for the real-time, sensitive and selective detection of energetic explosives in seawater. Three primary issues will be explored: 1) off-chip development of new methods for enhancing the sensitivity to explosives via a pre-concentration step from seawater into the nonaqueous solvent, acetonitrile; 2) on-chip implementation of ultra-miniaturized solid phase extraction (SPE); and 3) coupling of SPE to a high speed microchip separation platform using electrochemical detection.

APPROACH: The approach taken will be to first demonstrate the viability of performing rapid micro-extraction and concentration enhancement of energetic explosives such as TNT and dinitrotoluene (DNT) from seawater into acetonitrile. Micro-SPE columns will be prepared in either microbore capillary tubing or microchannels etched into planar microchip glass slides. The effort will be focused on dramatically reducing the necessary seawater sample size, while maintaining a high concentration factor into the eluting solvent, acetonitrile. Secondly, the speed and sensitivity of separating energetic explosives on a capillary electrophoresis (CE) microchip will be optimized, paying close attention to all relevant parameters, including the separation field strength, microchannel column length, pre-concentrator design and dimensions, electrode material and design, surface modification, the effect of a non-aqueous solvent, and scanning voltammetric techniques.

ACCOMPLISHMENTS: Three different off-chip extraction methods were assessed with respect to speed, efficiency and concentration factor for extracting explosives from seawater: packed micro-tubing (columns), extraction membranes and microextraction fibers. Because of their small surface area, the PDMS-DVB coated fibers were unable to achieve satisfactory concentration factors without utilizing unacceptably long adsorption and desorption times. The SDVB coated extraction membranes manufactured by 3M gave concentration enhancements as high as 50 times for TNT, when utilizing a 70 second seawater pumping time (6.5 ml/min). However, there was significant variability in extraction efficiency from membrane to membrane and the efficiency dropped off with each subsequent trial. For the packed micro-tubing approach, two column packing materials, Porapak and LiChrolut, were selected for study. For any given volume of seawater pumped through the microcolumn, LiChrolut outperformed Porapak with regards to the concentration enhancement of explosives and explosive derivatives. Concentration enhancements over 1000 times were possible for TNT. For the elution of explosives in acetonitrile, the highest concentration enhancement was obtained in the first 5 microliters of acetonitrile eluting off the column, and any increases in this volume of eluent resulted in decreasing the

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enhancement factor by dilution. This result indicates the importance of performing solid phase extraction (SPE) directly on the microchip.

On-chip SPE was examined utilizing three different materials: 1) C_{18} coated silica beads, 2) porous organic sol gel compositions, and 3) C_{18} coated paramagnetic beads. A special glass microchip was designed and microfabricated that permitted both the sampling and elution of preconcentrated analyte plugs, with subsequent delivery to a separation microchannel for separation and detection. All sampling was performed by electroosmotic flow across the packed bed. C_{18} coated silica beads were encapsulated within a microchannel bed using a porous poly(methacrylate) polymer grown inside the channel by UV photoinitiation. Results verify a linear increase in analyte preconcentration with increasing time of sampling across the microcolumn bed. Total column capacity was over 12 picomoles of Rhodamine B. Preconcentration factors ranged from 20 to 300 times, depending upon the time spent sampling across the bed, and detection limits for the test case dye, Rhodamine B, were obtained in the femtomolar range. We have also investigated the preparation of porous sol gel materials directly within the microchannel bed. Methyltrimethoxysilane (TMOS) has been used as the primary gel component, in conjunction with other alkyl-trimethoxysilanes containing a single alkyl chain that varies from C_3 - C_{18} . This material has great promise for high extraction efficiencies due to its incredibly high surface area, covalent linkage to the walls of the microchip, and capability for porosity and hydrophobicity control during preparation. Results have demonstrated that the hydrophobicity of even the TMOS derived sol gels is sufficient to give quantitative extraction of Rhodamine B onto the extraction bed. Finally, the C_{18} coated paramagnetic beads were investigated for their potential with regards to column preparation using an electromagnet positioned close to the microchannel bed, or column regeneration by removal of the electromagnet. Electroosmotic flow was utilized to deliver the paramagnetic beads down the microchip to the microchannel length directly above the applied magnetic field. Packed column beds were prepared, but the packing efficiency of these beds was relatively poor due to the non-spherical nature of the commercially available paramagnetic beads. In addition, as the bed size grew beyond approximately 1-2 mm in length, the applied magnetic field was no longer able to maintain the position of the extraction bed, and prevent its gradual migration down the channel. Finally, converting from aqueous to nonaqueous solvent resulted in a temporary but catastrophic reversal in fluid flow that disrupted the integrity of the packed bed.

The nonaqueous phase electrochemistry of explosives is critical to the successful coupling of an on-line SPE system with the microchip separation platform. When working with acetonitrile utilizing $LiClO_4$ as the supporting electrolyte, the addition of acetic acid was necessary in order to observe a TNT reduction peak. This can be ascribed to the fact that the electrochemical reduction of TNT requires the transfer of a proton. Interestingly, when the supporting electrolyte tetramethylammonium perchlorate was used, acetic acid addition was unnecessary for the observation of the TNT reduction peak by cyclic voltammetry. Finally, when a basic supporting electrolyte is utilized, such as tetraethylammonium hydroxide, TNT reacts irreversibly and rapidly to form a new, strongly colored pink derivative. In addition to the electrochemical activity of TNT observed in pure

acetonitrile solutions, we also investigated the electrochemical activity seen with increasing concentrations of buffered pH 8.7 borate water, the aqueous composition normally utilized in the electrochemical reduction of TNT. As the concentration of aqueous phase reaches 30%, the TNT reduction peak at -0.93 V clearly becomes visible, continuing to grow in as a series of three reduction peaks as the aqueous phase concentration increases.

Under basic, acetonitrile conditions, trinitroaromatic explosives such as TNT, TNB and tetryl, quickly react to form pink derivatives that absorb very strongly at 510 nm. Fourteen different explosive derivatives were examined for colorimetric reactions in basic acetonitrile, and only these three explosives gave colorimetric products at this probing wavelength. We can take advantage of this phenomenon by incorporating a 510 nm LED into the microchip design for allowing absorbance detection of these explosives as they are separated down the microchannel. On the microchip and in the absence of solid phase extraction, the detection limits obtained for the trinitroaromatic explosives were as follows: TNB- 60 $\mu\text{g/l}$; TNT- 160 $\mu\text{g/l}$, and tetryl- 200 $\mu\text{g/l}$. A mini-column was prepared from the solid phase extractant, Lichrolut, and utilized for preconcentrating the three trinitroaromatics from a 40 ml seawater sample spiked with 0.5 $\mu\text{g/l}$ of each trinitroaromatic into a 17 μl MeCN/MeOH nonaqueous sample for introduction to the microchip. The detection limits observed following SPE were as follows: TNT- 0.34 $\mu\text{g/l}$, TNB- 0.25 $\mu\text{g/l}$, and tetryl- 0.19 $\mu\text{g/l}$. Actual concentration enhancements observed are detailed as follows: TNT- 470 times, TNB- 240 times, and tetryl- 1050 times. The use of MeCN as both the eluting solvent as well as the running buffer for the CE microchip separation clearly offers a great advantage by avoiding sample dilution and consequent decreases in the overall concentration enhancements observed.

CONCLUSIONS: We have demonstrated successful direct coupling of micro-SPE enrichment with advanced separation techniques for model dye compounds. Efforts are underway to couple enrichment technologies with microseparation devices for the separation of nitroaromatic explosive mixtures, investigations which should ultimately lead to powerful detection schemes for explosives and other toxic analytes of concern to the DoD and analytical community alike.

SIGNIFICANCE: The sensitive and selective detection of explosives in oceanic environments is of critical importance to the Navy in their efforts to 1) remotely detect and locate underwater unexploded ordnance (UXO) for environmental cleanup and safety purposes, and 2) map out explosive signatures evident in plumes for target localization and identification purposes. The miniature size and rapid separation times evident on CE microchips make this new technology well suited for eventual integration into the REMUS Autonomous Underwater Vehicle (AUV) system utilized in chemical sensing sea explorations. Information gathered with respect to the development of a miniaturized, on-line solid phase extraction system will positively impact many different analytical approaches (e.g., IMS) currently being examined for the detection of explosives in seawater, due to the concentration enhancement observed, as well as the removal of the explosives from the troublesome seawater matrix.

PATENT INFORMATION: none

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